Review article

The assembly of triacylglycerol-rich lipoproteins: an essential role for the microsomal triacylglycerol transfer protein

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Raised plasma triacylglycerol is an independent risk factor for cardiovascular disease, and an understanding of factors which regulate the synthesis and degradation of lipoproteins which carry triacylglycerol in the blood may lead to novel approaches to the treatment of hypertriacylglycerolaemia. An active microsomal triacylglycerol transfer protein (MTP) is essential for the assembly of particles which transport triacylglycerol through the circulation. After absorption in the intestine, dietary fat and fat-soluble vitamins are incorporated into chylomicrons in the intestinal epithelial cells, and these lipoproteins reach the bloodstream via the lymphatic system. Patients with the rare genetic disorder, abetalipoproteinaemia, in which MTP activity is absent, present clinically with fat-soluble vitamin and essential fatty acid deficiency, indicating a key role for MTP in the movement of fat into the body. The triacylglycerol-rich lipoprotein found in fasting blood, VLDL, is assembled in the liver by an MTP-dependent process similar to chylomicron assembly, and transports triacylglycerol to extra-hepatic tissues such as adipose tissue and heart. In the absence of MTP activity, VLDL are not synthesized and only extremely low levels of triacylglycerol are present in the blood. Dietary components, including fat, cholesterol and ethanol, can modify the expression of the MTP gene and, hence, MTP activity. The present review summarizes current knowledge of the role of MTP in the assembly and secretion of triacylglycerol-rich lipoproteins, and the regulation of its activity in both animal and cell systems.

Triacylglycerols: Lipoproteins: Microsomal triacylglycerol transfer protein: Apolipoproteins: Abetalipoproteinaemia

The first description of what later came to be known as the microsomal triacylglycerol transfer protein (MTP) was made by Wetterau & Zilversmit (1984). This protein, which was originally isolated from bovine hepatic microsomal fractions (Wetterau & Zilversmit, 1985), was later shown to be present in the lumen of the endoplasmic reticulum of both liver and intestine in the rat (Wetterau & Zilversmit, 1986). Its structure differs from the previously described monomeric lipid transfer proteins such as the intracellular lipid transfer proteins (for review, see Rueckert & Schmidt, 1990) and plasma cholesteryl ester transfer protein (Drayna *et al.* 1987), in that it was a heterodimer in which the larger 97 kDa subunit had lipid transfer activity and the smaller subunit was identical to protein disulfide-isomerase (*EC* 5.3.4.1; PDI; Wetterau *et al.* 1990). Subsequently, Wetterau and his colleagues (Wetterau *et al.* 1997) have made a major contribution to our understanding of the structure and function of the MTP and, indeed, have recently published a detailed and comprehensive review.

The question might be asked as to why a review on MTP should be of interest to nutritionists. First, MTP is involved in the assembly of chylomicrons, and as such plays an important role in fat absorption from the gut. Second, its role in VLDL synthesis means it may play a critical role in regulating plasma lipoprotein concentrations. It may regulate VLDL concentration which, according to increasing evidence, might represent an independent risk factor for CHD (Griffin & Zampelas, 1995). Furthermore, as VLDL is the precursor particle for the synthesis of LDL, MTP may be

Abbreviations: ApoB, apolipoprotein B; cDNA, complementary DNA; HMGCoA, hydroxymethylglutaryl-CoA; IC50, inhibitory concentration giving 50 % maximal activity; MTP, microsomal triacylglycerol transfer protein; PDI, protein-disulfide isomerase.

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important in regulating the concentration of this well-established risk factor.

Over the past few years, because of its potential importance in regulating lipoprotein metabolism, increasing interest has focused on the manipulation of MTP activity. To this end, a number of pharmaceutical companies have developed, or are developing, drugs which inhibit MTP activity. It has also become apparent that diet may play an important role in regulating its activity. The aim of the present review is to provide an overview of MTP structure and function, and to describe in more detail the potential regulation of its expression by dietary constituents.

The structure of microsomal triacylglycerol transfer protein

As mentioned previously, MTP is a heterodimeric protein in which the smaller subunit is identical to PDI, itself the beta-subunit of prolyl 4-hydroxylase (*EC* 1.14.11.2), the vitamin C-dependent enzyme responsible for the hydroxylation of collagen (Pihlajaniemi *et al*. 1987). The close association between the two subunits in MTP was illustrated using antibodies to either subunit which, when added to MTP in solution, removed both subunits and triacylglycerol transfer activity (Wetterau *et al*. 1990). Structural analysis confirmed the strong association of the two subunits and their presence in a ratio of 1:1 in the mature protein (Wetterau *et al*. 1991*a*). Dissociation of the MTP complex under mild non-denaturing conditions led to a loss of transfer activity and aggregation of the larger subunit, indicating its hydrophobic nature, and suggested that PDI is required specifically to maintain a functionally-active MTP (Wetterau *et al*. 1991*b*). Catalytically-active PDI, however, is apparently not required for MTP activity, since co-expression of the large MTP subunit in insect cells together with an artificially-mutated form of PDI, in which the catalytic sites were inactivated, gave rise to soluble dimeric MTP which exhibited lipid transfer activity comparable with that of the wild-type protein (Lamberg *et al*. 1996). In this respect, PDI appears to be acting in a manner similar to its role in prolyl 4-hydroxylase where, as the beta-subunits, its catalytic isomerase activity is not required for tetramer assembly or hydroxylase activity, but the protein is critical for maintaining the α-hydroxylase subunits in a catalytic, non-aggregated conformation (Vuori *et al*. 1992). A further important role of PDI may be to ensure the retention of the MTP complex in the lumen of the endoplasmic reticulum, since PDI possesses the C-terminal tetrapeptide sequence, KDEL (Vuori *et al*. 1992), which the large MTP subunit lacks. This KDEL sequence (Lys-Asp-Glu-Leu) is common to proteins which are retained in the rough endoplasmic reticulum. The complementary DNA (cDNA) which encodes the human MTP large subunit was isolated and sequenced by Sharp *et al*. (1993). It exhibits 88 % homology with both bovine (Sharp *et al*. 1993) and hamster (Lin *et al*. 1994) MTP cDNA. Subsequently, the gene encoding the large subunit of MTP was characterized by the same group (Sharp *et al*. 1994) and localized to band 4q24 of chromosome 4 by fluorescent *in situ* hybridization. It consists of eighteen exons and spans 55–60 kb. The mouse (Nakamuta *et al*. 1996) and chicken (Ivessa *et al*. 1996) MTP large subunits have also recently been cloned. The cDNA encodes a polypeptide of 894 amino acids with a molecular weight of 99 kDa for the nascent protein, which loses its signal sequence during processing to the mature 97 kDa polypeptide. This signal sequence is presumably required for the co-translocational insertion of apolipoprotein B (apoB) into the inner leaflet of the endoplasmic reticulum (Pease *et al*. 1991). Studies of the characteristics of lipid binding to the large subunit and the mechanism of lipid transfer (Atzel & Wetterau, 1993, 1994) identified two separate lipid binding sites, fast and slow. Kinetic analysis of lipid transport indicated that MTP shuttles both triacylglycerol and cholesteryl ester between membranes using the fast sites. The ability of MTP to transfer lipids correlates strongly with the binding of the specific lipids to the protein, with non-polar lipids being preferred (Jamil *et al*. 1995). The main features of MTP structure and function are summarized in Table 1.

Abetalipoproteinaemia

Abetalipoproteinaemia is a rare autosomal recessive dyslipidaemia characterized by the absence of apoB-containing lipoproteins in the circulation, and consequently almost undetectable plasma triacylglycerols and very low plasma cholesterol, all of which is associated with an HDL fraction (Gregg & Wetterau, 1994). The major clinical consequences of the disorder (Table 2) may be ascribed to essential fatty acid and fat-soluble vitamin deficiency, particularly vitamins A and E. Chylomicrons play a critical role in the transport of these fat-soluble vitamins from the enterocyte into the lymphatic system and eventually into the blood. Thus, although fats, including fat-soluble vitamins, are absorbed into the enterocytes of abetalipoproteinaemic patients, they cannot be incorporated into chylomicrons, and so the enterocytes become engorged with fat. Chronic diarrhoea ensues if a normal fat-containing diet is continued. Often patients self-compensate

Table 1. Microsomal triacylglycerol transfer protein (MTP): function, structure and expression

Function
Facilitates assembly and secretion of apoB-containing lipoproteins (chylomicrons in the intestine and VLDL in the liver) by transfer of triacylglycerol, cholesteryl ester and phospholipid to nascent apoprotein
Structure
Heterodimer:
59 kDa protein disulfide isomerase (*EC* 5.3.4.1)
97 kDa unique large subunit, with extensive homology to amphibian *Xenopus laevis* lipovitellin, a lipid-binding protein synthesized in the liver and found in the egg
Expression of MTP large subunit
Gene of 55–60 kb, eighteen exons, seventeen introns, on human chromosome 4(q24) Expressed mainly in the endoplasmic reticulum of intestine and liver
Expression of MTP increased by:
Dietary saturated fatty acids
Dietary cholesterol
Expression of MTP decreased by:
Insulin
Ethanol consumption

Table 2. Abetalipoproteinaemia*

A condition caused by the absence of MTP activity
Rare autosomal recessive disease
Chronic diarrhoea, secondary to fat malabsorption
Fat-filled enterocytes and parenchymal hepatocytes
Fat-soluble vitamin deficiency
Abnormal erythrocytes (acanthocytosis)
Atypical retinitis pigmentosa
Nightblindness, loss of colour vision
Ataxia, absence of deep tendon reflex
Atypical spinal-cerebellar degeneration
Absence of apoprotein B (apoB)-containing lipoproteins in circulation (VLDL and LDL) and very low concentration of circulating triacylglycerols

* The classical recessive form of abetalipoproteinaemia should be distinguished from the co-dominant hypobetalipoproteinaemia, which results from mutations in the apoB gene, leading to truncated forms of apoB protein. Whereas homozygous abetalipoproteinaemia and hypobetalipoproteinaemia patients have virtually identical circulating lipid levels, parents of abetalipoproteinaemic patients (heterozygotes) have normal lipid levels, while heterozygous parents of hypobetalipoproteinaemic patients have an approximately 50 % reduction in apoB-containing lipoprotein levels.

for this by eating diets with very low or no fat content. This only exacerbates the situation, and patients invariably present with loss of colour vision and nightblindness. Unless alternative methods of administration of fat-soluble vitamins and essential fatty acids are available, the patients become wheelchair bound by the third decade of life, and usually die by the age of 40 years.

The finding that both MTP activity and the large subunit of MTP were absent in intestinal biopsies taken from four patients with abetalipoproteinaemia provided strong evidence for defects in MTP as the underlying cause for the disease, and also for a role of MTP in the assembly of triacylglycerol-rich lipoproteins (Wetterau *et al*. 1992). Confirmation of this conclusion came from comparisons of the genomic sequence of MTP isolated from homozygous abetalipoproteinaemic patients with cDNA from control subjects (Sharp *et al*. 1993; Shoulders *et al*. 1993). In each case, mutations in the gene encoding the large subunit of MTP were found which gave rise to a non-functional MTP. Thus, the disease is not one of defective lipid or apoB synthesis, but of defective lipoprotein assembly for which an active MTP is a critical requirement.

The assembly of triacylglycerol-rich lipoproteins

The synthesis and secretion of apoprotein B

This section will deal primarily with the synthesis and secretion of VLDL, since model systems are readily available for study. However, it is likely that the synthesis and secretion of chylomicrons by the intestinal epithelium follow the same route, although their regulation by diet and hormones in this tissue is different from that in the liver. A description of the role of MTP in the cell requires the inclusion of some information on the synthesis of apoB and its movement through the cell. In the following discussion the size of a particular truncated apoB molecule is denoted on the centile scale, where the full-length mature protein synthesized in the liver is termed apoB100. Thus, apoB32, for example, indicates an apoB molecule containing the N-terminal 32 % of the mature protein, i.e. lacking the C-terminal 68 %. The primary apoprotein component of triacylglycerol-rich lipoproteins is apoB, apoB100 in VLDL and apoB48 in chylomicrons. ApoB, like other secretory proteins, has a signal sequence at its amino terminal which ensures its translation on ribosomes bound on the endoplasmic reticulum. The subsequent translocation into the lumen of the endoplasmic reticulum is dependent on a supply of lipid to the nascent protein, and in the absence of lipid, apoB remains membrane-bound and is degraded (Davis *et al*. 1990). There is thus competition for apoB between assembly into VLDL and degradation. Borchardt & Davis (1987) showed that much of the newly-synthesized apoB in hepatic cells was not secreted but degraded intracellularly, and that translocation across the membrane of the endoplasmic reticulum was inefficient. This was due to the generation of a translocation-arrested intermediate of apoB, in which the N-terminal 69 kDa portion projected into the lumen of the endoplasmic reticulum, while the remaining C-terminus was on the cytosolic surface of the endoplasmic reticulum (Du *et al*. 1994). Any apoB which is not fully translocated into the lumen of the endoplasmic reticulum undergoes proteolytic cleavage on the cytosolic surface by an N-acetyl-leucyl-leucyl-norleucinal-sensitive protease, releasing an 87 kDa N-terminal fragment of apoB into the lumen. This fragment is then secreted via the normal default pathway, and may be present in the circulation. Subsequently, Bonnardel & Davis (1995) showed that translocation rather than degradation determined the fate of apoB. ApoB which enters the lumen of the endoplasmic reticulum also follows the common secretory route (the default or anterograde pathway) to the plasma membrane via coated vesicle trafficking (Rustaeus *et al*. 1995). The special feature of apoB is that it acquires lipid, predominantly triacylglycerol but also cholesteryl ester and phospholipid, as it passes through the secretory pathway and is secreted as a lipid-rich lipoprotein particle.

The role of microsomal triacylglycerol transfer protein in triacylglycerol-rich lipoprotein assembly

The absolute requirement for the role of MTP in this process is shown *in vivo* by the clinical condition of abetalipoproteinaemia described previously, and from experiments in primary hepatocytes, hepatoma cells and heterologous cells, in which apoB and MTP have been expressed. Thus, transfection of C-terminally truncated apoB into cells which do not normally synthesize lipoproteins, for example apoB41 in a monkey kidney cell line (COS-1, see Appendix; Leiper *et al*. 1994) and apoB53 in HeLa cells (Gordon *et al*. 1994), did not lead to secretion of apoB unless MTP was co-transfected into the cells. In the absence of MTP the truncated apoB molecules were expressed, but then degraded intracellularly rather than secreted. Furthermore, secretion in the presence of co-transfected MTP was increased by supplying the cells with an exogenous source of lipid as oleate. Similar results from transfection experiments with truncated apoB (larger than apoB29) in COS cells (Patel & Grundy, 1996) and human apoB48 in rat hepatoma cells (Wang *et al*. 1997), including in the latter work the use of a specific photo-affinity inhibitor of MTP,

further confirmed that MTP was necessary and sufficient to direct the regulated secretion of apoB in the presence of a lipid supply.

An insight into the precise role of MTP in the assembly of triacylglycerol-rich lipoproteins has been gained from a number of studies which have sought to dissect particular aspects of the process, again using both hepatic and heterologous cells transfected with C-terminal truncated apoB and the large subunit of MTP. Deductions from the results of such studies, of course, assume that what applies to truncated apoB is also true for the full-length mature protein.

From experiments with apoB28 expressed in HepG2 and COS-1 cells, Ingram & Shelness (1997) showed that folding of the amino terminal disulfide-bonded domain of apoB is likely to be a co-translational event occurring soon after translocation of this domain into the endoplasmic reticulum lumen. This initial folding did not require MTP activity. However, folding of this N-terminal domain was essential for the MTP-dependent association of the nascent protein with lipid, and suggests that initiation of lipoprotein formation in the endoplasmic reticulum takes place in two distinct steps: (1) co-translational folding of the amino terminal globular domain of apoB, followed by (2) MTP-dependent association of lipid with sequences further along the peptide chain, which is facilitated by the initial protein folding. The second of these steps, which is critical to the fate of apoB, may begin when the nascent protein has reached a size of about 80 kDa (Boren *et al*. 1994). Although transcriptional control of the apoB gene and, hence, apoB synthesis has been demonstrated (Bennett *et al*. 1995), it is thought that hepatic secretion of apoB is to a large extent regulated post-transcriptionally. Addition of lipid at the appropriate times is one such level of control. A number of proteolytic activities directed towards apoB exist throughout the default pathway, and appear to act as a quality-control mechanism to ensure the synthesis of secretory-competent VLDL particles. Cartwright & Higgins (1996) showed that in rabbit hepatocytes intracellular degradation of apoB100 occurred in the endoplasmic reticulum and *trans*-Golgi membranes (see Appendix), and involved a number of different proteases. ApoB that accumulated in the endoplasmic reticulum lumen, and presumably became associated with lipid, was destined for secretion, while apoB that accumulated in *trans*-Golgi membranes was irretrievably directed away from secretion. Wang *et al*. (1996) used non-hepatic COS-7 cells transfected with genes encoding C-terminally truncated forms of apoB with or without co-expression of the large subunit of MTP, and showed that the MTP decreased co-translational degradation of apoB probably by assisting its translocation into the endoplasmic reticulum lumen, thereby promoting VLDL assembly and secretion.

The availability of intracellular triacylglycerol seems to be critical for the stimulatory effect of MTP on VLDL secretion. For example, transfection of apoB and MTP in insect cells, which do not normally support lipoprotein synthesis and secretion, did not cause any significant increase in apoB secretion unless intracellular triacylglycerol concentrations were raised by addition of a source of fatty acid such as oleate to the culture medium (Gretch *et al*. 1996). The addition of lipid to apoB during assembly of the lipoprotein particle appears to occur in two quite distinct phases, involving the co-translational production of a lipid-poor lipoprotein precursor, followed by the addition of the bulk lipid to this precursor. There is good evidence for a role of MTP in the first stage, but there is uncertainty about a possible role in the second (Gordon *et al*. 1996). VLDL secretion by the rat hepatoma cell McArdle-RH7777 was stimulated by addition of oleate to the culture medium. Treatment of these cells with an MTP photo-affinity inhibitor before pulse labelling with ^{35}S-labelled amino acids (methionine and cysteine) blocked the secretion of radiolabelled apoB by 85 %. However, when cells were pulse labelled with ^{35}S-labelled amino acids (methionine and cysteine) and allowed to form the MTP-dependent lipid-poor radiolabelled lipoprotein precursor before addition of the MTP inhibitor, there was no inhibition of ^{35}S-labelled apoB-containing VLDL secretion on addition of oleate to the medium. The conclusion drawn was that MTP was involved in the production of the initial lipid-poor lipoprotein precursor particles, but was not required for the addition of the bulk lipid to the particle. Also, the results confirmed that the action of MTP is dependent on a supply of intracellular triacylglycerol, provided in this case by oleate. Such a two-step mechanism for lipid addition during VLDL assembly is supported by the results of Cartwright *et al*. (1997), who determined the distribution of newly-synthesized lipid in microsomes derived from the endoplasmic reticulum of adult rabbit hepatocytes. As apoB moves into the lumen of the rough endoplasmic reticulum it is accompanied by only small amounts of cholesteryl ester, cholesterol and triacylglycerol. The addition of the bulk of these lipids occurs in the smooth endoplasmic reticulum, and it is this addition which is stimulated by exogenous fatty acid. The apparent absence of a requirement for MTP activity for the transfer of bulk lipid in the assembly of the VLDL particle, of course, begs the question as to what is the nature of this process. Subcellular fractionation of hamster liver indicates that MTP, detected immunologically, was present throughout the rough and smooth endoplasmic reticulum, as well as in the *cis*-Golgi and possibly also the *trans*-Golgi (J Higgins, personal communication), so perhaps it is too early to say that MTP plays no role in bulk lipid addition to the maturing lipoprotein particle. In the inhibitor studies described previously the inhibition of MTP activity was approximately 70 %, and it might be that the remaining activity is sufficient to account for lipid transfer, particularly if the characteristics of the transfer process were altered. The studies of Gordon *et al*. (1996) were concerned with the secretion of apoB48-containing lipoproteins. However, van Greevenbroek *et al*. (1998), using the MTP inhibitor described by Jamil *et al*. (1996), concluded that in Caco-2 cells MTP was also involved in bulk triacylglycerol addition, as well as the first step described previously during the assembly of apoB100-containing lipoproteins. Furthermore, Gordon (1997) does not rule out a post stage one subsidiary role for MTP in modifying the triacylglycerol-rich lipoprotein secretion without substantially changing their composition.

Structural requirements for MTP function

The efficient transfer of lipid from MTP to the nascent apoB molecule has specific structural requirements for both apoB and MTP. An intact C-terminus of MTP is an absolute requirement. The mutant MTP genes isolated from eight abetalipoproteinaemic patients who had mutations in both alleles were predicted to encode truncated forms of MTP with variable numbers of aberrant amino acids at their C-termini (Narcisi *et al*. 1995). Furthermore, expression of genetically-engineered MTP in COS-1 cells indicated that deletion of the last twenty amino acids from the C-terminus of the 894 amino acid protein, or a mutation changing residue 878 from cysteine to serine, both abolished MTP activity. Similarly, Ricci *et al*. (1995) isolated and sequenced another human mutant MTP gene containing a nonsense mutation which predicted an altered MTP, C-terminally truncated (by thirty amino acids). Co-expression of this mutant MTP with PDI in insect cells Sf9 (see Appendix) led to high expression of the mutant MTP, but unlike the normal MTP, the mutant protein was unable to form a stable, soluble complex with PDI, and was hence non-functional. A further mutant MTP gene product, which contained an Arg 540 His missense mutation (see Appendix), was similarly unable to form a stable complex with PDI, and was consequently non-functional and unable to support apoB secretion (Rehberg *et al*. 1996).

Structural requirements of apoprotein B for its incorporation into VLDL

In experiments designed to determine which parts of apoB are required for interaction with MTP, Gretch *et al*. (1996) expressed MTP and nine segments, including the amino terminal, internal and carboxy terminal regions, of the apoprotein into insect cells. Oleate-induced secretion was found for all segments containing the amino terminal 17 % of apoB, apart from the segment containing only this first 17 %, indicating that the amino terminal part of apoB is required but not inherently sufficient for accepting lipid from MTP. There also appears to be a minimum size requirement for apoB to be secreted as part of VLDL, since expression of various C-terminally truncated apoB by rat hepatoma cells indicated that truncation to apoB34 or less diminished or completely abolished VLDL secretion (McLeod *et al*. 1996). By constructing chimaeric proteins consisting of the human apolipoprotein apoA-I (normally found in HDL) and various segments of apoB between apoB29 and apoB42, the same authors showed that a number of sequences, 153–237 amino acids in length, were capable of binding lipid and being secreted as VLDL-like particles. Some of these short hydrophobic sequences may facilitate the recruitment of the initial lipid as well as the bulk lipid during the assembly of hepatic VLDL. However, it is not known if the recruitment of triacylglycerol to any of these sites (i.e. between apoB29 and apoB42) is dependent on the action of MTP.

The transfer of lipid from MTP to apoB involves a direct physical association between the two proteins, and this complex is stable to high-salt washing and also low pH, implying a strong hydrophobic interaction (Patel & Grundy, 1996). Such an interaction was also shown by Wu *et al*. (1996), who used an anti-MTP antiserum to immunoprecipitate lysates from cells which had been labelled with [^{3}H]leucine and were actively secreting VLDL. Analysis of the immunoprecipitate revealed radiolabelled apoB, PDI and the large subunit of MTP. Kinetic analysis indicated that the association of MTP with apoB, which was particularly evident early in apoB transport, was transient but more prolonged when there was an increased supply of triacylglycerol.

Regulation of microsomal triacylglycerol transfer protein

Gene transcription is usually regulated by interaction of proteins, termed transcription factors, with specific DNA sequences in a region termed the promoter, near the site where transcription begins. Molecular studies of the promoter region of the MTP gene indicate a number of potential sites to which specific transcription factors may bind and regulate gene expression. The promoter has been found to contain consensus DNA sequences for binding of transcription factors, hepatic nuclear factors-1 and -4 and activator protein-1 (Hagan *et al*. 1994). Hepatic nuclear factors-1 and -4 are primarily expressed in liver, with some expression in intestine and kidney, and regulate the expression of a number of liver-specific genes. Hepatic nuclear factor-4 is a member of the steroid hormone receptor super family of transcription factors, some members of which have been found to respond to fatty acids. This raises the interesting possibility of regulation of MTP by lipids. Also present in the promoter are a negative insulin response element and a modified sterol response element. The former suggests that the expression of the MTP gene may be regulated by insulin, and the latter, which is related to a response element found in a number of genes coding for proteins involved in lipoprotein metabolism, including the LDL receptor, hydroxymethylglutaryl-CoA (HMGCoA) reductase (*EC* 1.1.1.88), HMGCoA synthase (*EC* 4.1.3.5) and cholesterol 7α-hydroxylase (*EC* 1.14.13.17; Hagan *et al*. 1994), suggests that cholesterol may also play a role in its regulation. To investigate whether these response elements are active in the MTP gene, Hagan *et al*. (1994) linked the MTP promoter to a luciferase gene. The construct was then transfected into cells, and expression of the luciferase 'reporter gene' was investigated. As expected, insulin down-regulated expression of the gene but, more surprisingly, cholesterol increased expression. This is the opposite effect to that seen with related sterol response elements found on the LDL receptor, HMGCoA synthase or HMGCoA reductase promoters. However, cholesterol has been found to increase the expression of the cholesterol 7α-hydroxlase gene, whose promoter also contains a potential sterol response element (Hagan *et al*. 1994).

Effects of insulin and cholesterol on the expression of the endogenous MTP gene in cells in culture have been less clear cut. Lin *et al*. (1995) showed that insulin caused a dose- and time-dependent decrease in MTP mRNA, and that this effect was mediated through the insulin receptor. However, because of the long half-life of the MTP protein, this was not accompanied by any change in MTP protein concentration or activity. The effect of cholesterol

on MTP expression in cultured cells has been described as minimal, although this was attributed to the insensitivity of the method for determining RNA concentration (Hagan *et al*. 1994).

Regulation of microsomal triacylglycerol transfer protein in vivo

It is clear from the previous discussion that a number of potentially important regulatory sites have been identified within the promoter of the MTP gene. Clearly, it is important to show whether these have any physiological importance *in vivo*. Such experiments are difficult to perform using human subjects, since ethical considerations limit the availability of human intestine and/or liver biopsy samples. Thus, such work has been carried out using animal models. Apart from the studies described later using diabetic rats, the species of choice has been the Golden Syrian hamster. We, (Salter & White, 1996) and others (Dietschy *et al*. 1993) have shown this species to be very useful in studying the influence of diet on lipoprotein metabolism. Hamsters carry an appreciable amount of cholesterol in the LDL fraction, exhibit a hepatic cholesterol synthesis rate which is comparable with that of human subjects, and under appropriate dietary conditions will even develop atherosclerotic lesions.

Effect of insulin

As described previously, the MTP promoter contains a negative insulin response element which appears to be active in cultured cells. A possible role of insulin *in vivo* has been investigated using streptozotocin-diabetic rats. Brett *et al*. (1995) found that despite a markedly reduced VLDL output, no change in hepatic MTP activity could be detected. By contrast, Wetterau *et al*. (1997) report a 65 % increase in hepatic MTP mRNA concentrations in streptozotocin-treated animals, whereas no changes in intestinal MTP mRNA were observed. While this may be predicted from the cell culture studies, it obviously could not explain the decrease in VLDL production described by Brett *et al*. (1995). It appears that, at least under conditions where VLDL secretion is markedly reduced, i.e. in streptozotocin-diabetic rats and 10-d-old sucking rats, VLDL secretion is regulated at a level other than MTP. It has been suggested that the difference in results obtained in these two studies results from the different time period for which the animals were diabetic. Brett *et al*. (1995) measured MTP activity 5 d after treatment, while Wetterau *et al*. (1997) waited for 8 d. As the half-life of the protein has been described as 4·4 d, at least in HepG2 cells, it may be that only in the latter study had sufficient time elapsed to see a change.

Effect of dietary cholesterol

The MTP promoter has also been reported to contain a sterol response element. To investigate whether dietary cholesterol influences MTP concentrations, hamsters were fed on diets containing 0·05, 1·2 or 2·4 g cholesterol/kg for 4 weeks (Bennett *et al*. 1996). At the end of the feeding period, hepatic MTP mRNA concentrations were determined by nuclease protection assay, and levels were correlated with plasma VLDL-lipid concentrations. With increasing amounts of cholesterol in the diet, both VLDL-cholesterol and -triacylglycerol rose. This was associated with an increase in hepatic MTP mRNA, and highly significant correlations were seen between MTP mRNA and VLDL-lipid concentrations. In addition, MTP mRNA concentrations were also correlated with hepatic cholesteryl ester concentrations. It may be speculated that the increase in MTP gene expression represents part of a coordinated response to mobilize the increased amounts of cholesteryl ester which are accumulating in the liver. We have previously shown that feeding cholesterol also induces the activity of the enzyme phosphatidate phosphohydrolase (*EC* 3.1.3.4), believed to be rate-limiting in triacylglycerol synthesis (Sessions *et al*. 1993). Thus, an increased availability of triacylglycerol accompanied by increased ability to transfer it to apoB, through increased MTP activity, may enable the liver to also incorporate greater amounts of cholesterol into the newly-synthesized VLDL particles.

These findings clearly support the possibility that cholesterol may directly regulate MTP gene expression through the modified sterol response element in its promoter. However, other less-direct effects cannot be ruled out until more definitive evidence is available.

Effect of dietary fat

One of the first demonstrations of *in vivo* regulation of MTP expression was by Lin *et al*. (1994). Hamsters were fed on various diets for 31 d, including a control low-fat high-complex-carbohydrate diet, a high-saturated-fat diet (45 % of energy as hydrogenated coconut oil) and a high-sucrose diet. The high-fat and high-sucrose diet groups had considerably elevated hepatic MTP mRNA concentrations compared with the control group. Levels of intestinal mRNA were also elevated in the high-fat group. While the increase in intestinal MTP mRNA was evident after 24 h of feeding, the effect on hepatic mRNA was more chronic. We have subsequently shown that the response of hepatic MTP mRNA to dietary fat is dose-dependent, and varies depending on the nature of the fat. Increasing the amount of a mixture of triolein and tripalmitin (added in equal proportions) from approximately 22 % of total energy up to 44 % of total energy produced a linear increase in hepatic mRNA concentrations (Bennett *et al*. 1995). Interestingly, under these conditions, hepatic MTP mRNA concentrations showed significant positive correlation with total plasma cholesterol, VLDL-cholesterol, LDL-cholesterol and HDL-cholesterol, but not with total plasma triacylglycerol or VLDL-triacylglycerol. Similarly, in the study described previously (Lin *et al*. 1994), hepatic mRNA concentrations also correlated positively with HDL-cholesterol but not VLDL- + LDL-triacylglycerol levels. Considering the role of MTP in VLDL assembly, these results are somewhat surprising and, as yet, remain unexplained. The type of fat was also found to be important, with diets enriched in triolein alone or triolein together with linoleic acid-rich safflower oil producing lower levels of hepatic MTP mRNA than those enriched with mixtures of triolein and saturated fatty acid-rich fats such as tripalmitin and trimyristin

(Bennett *et al*. 1955). These results suggest a specific effect of saturated fatty acids in increasing hepatic MTP mRNA concentrations.

The precise mechanisms whereby dietary saturated fat influences MTP mRNA concentrations remain to be established. However, one possibility is a direct modulation of gene expression by fatty acids. For example, the MTP promoter is known to contain consensus sequences for the binding of the transcription factor HNF-4 and, as already discussed, HNF-4 may respond directly to fatty acids. However, as with the cholesterol response, definitive evidence is still lacking.

Effect of ethanol

In the light of the well-documented hypertriacylglycerolaemia induced by both acute and chronic ethanol consumption in human subjects, the recent observation of Lin *et al*. (1997) that a single oral dose of ethanol to rats caused a reduction in both intestinal and hepatic MTP mRNA levels is unexpected. Similar effects of ethanol on MTP mRNA levels were observed in HepG2 cells, and further analyses suggested that this was due to a decreased rate of MTP gene transcription. It may be that an inhibitory effect of ethanol on triacylglycerol-rich lipoprotein clearance may override any effect on lipoprotein synthesis and secretion.

Inhibitors of microsomal triacylglycerol transfer protein

Three inhibitors (Fig. 1) of MTP activity have been described in the literature, all of which cause a decrease in apoB secretion in cells in culture and inhibit MTP-mediated triacylglyercol transfer in liposome systems. 2-[1-(3,3-Diphenylpropyl)-4-piperidinyl]-2,3-dihydro-1H-isoindol-1-one (BMS-200150; Bristol–Myers–Squibb, Princeton, NJ, USA; Jamil *et al*. 1996) inhibited lipid transfer in an *in vitro* system in which the inhibitory concentration giving 50 % maximal activity (IC50) was 0·6 mM, a concentration similar to the dissociation constant for binding to the protein, suggesting that the inhibition of transfer is a direct result of the interaction of the inhibitor with MTP. BMS-200150 also caused a dose-dependent inhibition of apoB secretion from HepG2 cells (Jamil *et al*. 1996). The other two inhibitors are derivatives of the anticonvulsive and hypnotic drug methaqualone: 4′-bromo-methaqualone (CP-10477; Pfizer, Groton, CT, USA; Haghpassand *et al*. 1996) and 4′-bromo-3′-methylmetaqualone (Glaxo Wellcome, Les Ulis, France; Benoist *et al*. 1996). An extensive study with CP-10477 showed that the drug had no effect on triacylglycerol, protein synthesis or secretion of total protein, including albumin in HepG2 cells. However, it inhibited secretion of triacylglycerol and apoB by both HepG2 and CaCo2 cells (Haghpassand *et al*. 1996). Under conditions where apoB secretion was inhibited, apoB degradation in the early endoplasmic reticulum was stimulated. The drug also inhibited human liver MTP, but not cholesteryl ester transfer protein activity, *in vitro*. Benoist *et al*. (1996) reported independently on the similar properties of 4′-bromo-3′-methylmetaqualone; i.e. inhibition (IC50, 0·9 μM) of MTP-mediated but not cholesteryl ester transfer protein-mediated lipid transfer in an *in vitro* system, and inhibition of apoB100 (IC50, 0·3 μM) but not albumin secretion in HepG2 cells. Again inhibition of MTP activity led to increased degradation of apoB100, an effect which was completely reversed by previous incubation of the cells with dithiothreitol. It was proposed that transfer of lipid to

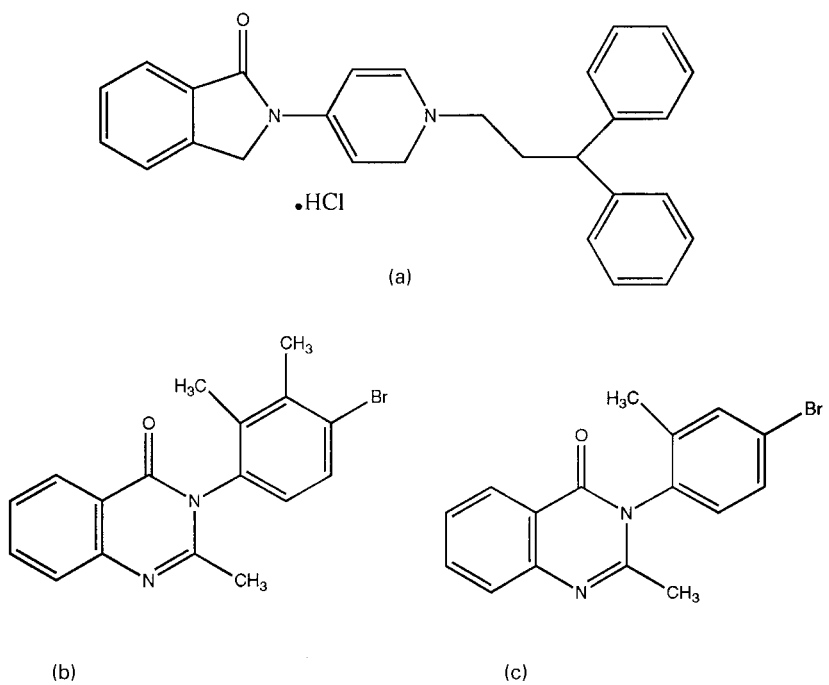

Fig. 1. Structures of pharmacological inhibitors of microsomal triacylglycerol transfer protein activity. (a) BMS-200150 (Jamil *et al.* 1996); (b) 4′-bromo-3′-methylmetaqualone (Benoist *et al.* 1996); (c) 4′-bromo-methaqualone (CP 10447; Haghpassand *et al.* 1996).

the nascent apoB *in vivo* prevents the early presecretory degradation of apoB by a dithiothreitol-sensitive protease.

Conclusions

A working model for the role of MTP in triacylglycerol-rich lipoprotein assembly may be developed from the previous discussion (Fig. 2). mRNA for apoB is bound by ribosomes in the cytoplasm and, after translation of the apoB signal sequence, the mRNA–ribosome–nascent partially-translated protein complex moves to the rough endoplasmic reticulum. Further translation and translocation of the protein into the lumen of the endoplasmic reticulum then proceeds. An element of control is exerted here and plays a critical role in determining the fate of the newly-synthesized apoB. Translocation of apoB will not continue unless first, the N-terminus folds correctly, presumably under the action of a chaperone protein such as heat shock protein 70 or BiP (Zhou *et al*. 1995), and second, lipid is transferred to the nascent protein through interaction with MTP. In the absence of either of these events, further translocation of apoB sequences into the rough endoplasmic reticulum lumen is blocked, so that the majority of nascent apoB polypeptide is degraded intracellularly, with only the already translocated N-terminus being secreted. Recent evidence suggests that this occurs by conjugation of apoB with ubiquitin, and degradation via the proteosome pathway (Benoist & Grand-Perrett, 1997; Fisher *et al*. 1997). When both these conditions are fulfulled, fully-translocated apoB, as a lipid-poor particle, follows the default pathway, undergoing post-translational modification in the Golgi and also accumulating more lipid, which may not be transferred by MTP. Finally, apoB is secreted as a component of a mature lipoprotein, a chylomicron in the intestine or VLDL in the liver. A number of proteolytic activities against apoB exist throughout the secretory pathway, which acts as a

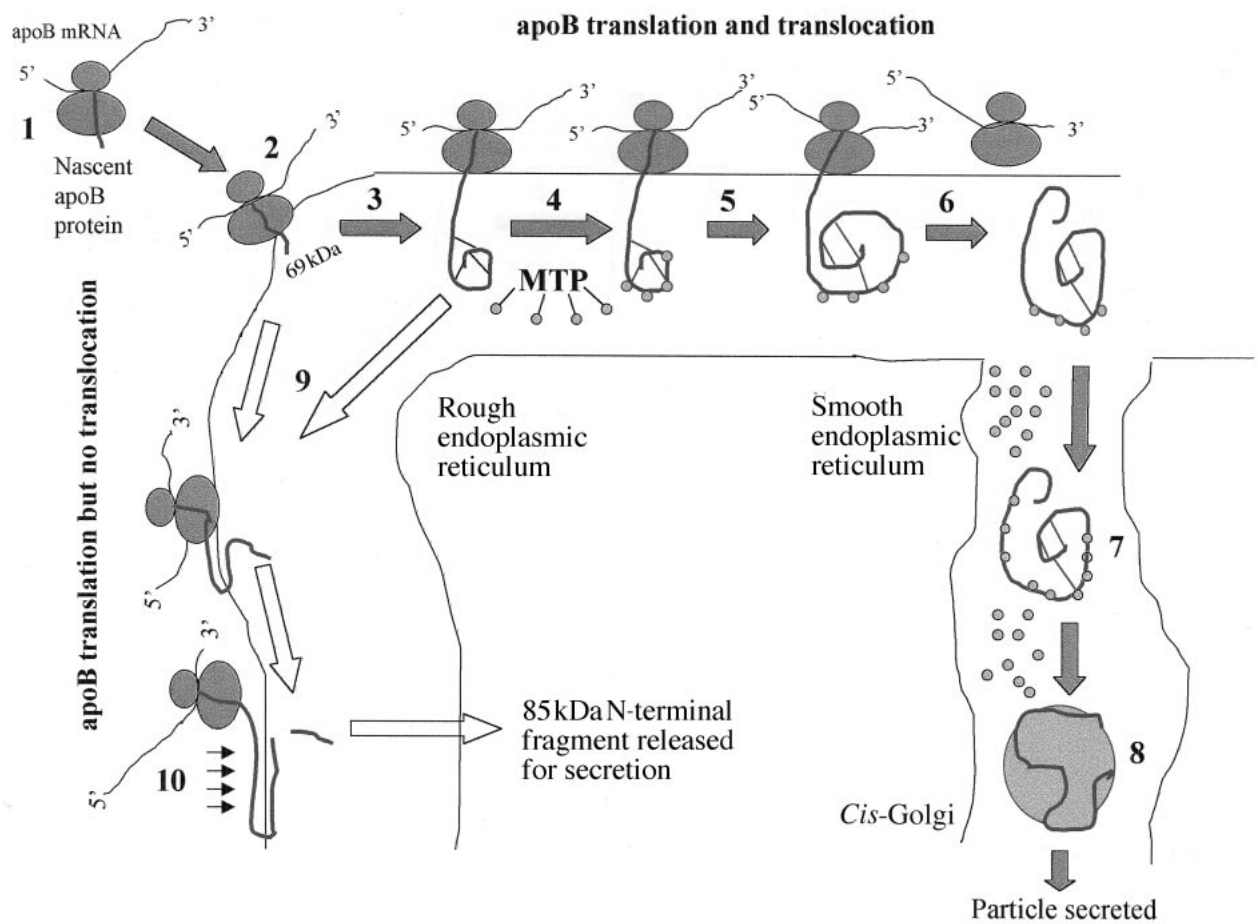

Fig. 2. The role of microsomal triacylglycerol transfer protein (MTP) in triacylglycerol-rich lipoprotein assembly. (1) Initiation of translation of apoprotein B (apoB) mRNA in the cytosol and translation arrest through interaction with the signal recognition particle. (2) Translation and translocation of apoB into the rough endoplasmic reticulum lumen; translocation arrested when N-terminal 69 kDa portion is in the lumen. (3) Folding of N-terminal (17 %) portion of apoB probably with the aid of chaperone proteins such as BiP, heat shock protein 70 and protein disulfide isomerase. (4) Addition of triacylglycerol and cholesteryl ester to N-terminal region by MTP. (5) Continued translation of apoB mRNA and translocation of apoB into the lumen. (6) Formation of a lipid poor, 'primordial' lipoprotein. (7) Addition of bulk lipid to the lipid-poor lipoprotein, probably not involving MTP. (8) Assembly of a secretory competent triacylglycerol-rich lipoprotein. The alternative metabolic route for apoB involves degradation and is taken if either steps 3 or 4 fail to occur. (9) Continued translation of apoB mRNA but arrested translocation of the protein. (10) Degradation of the C-terminal portion of apoB via a battery of proteolytic activites including ubiquitinylation and degradation by proteasomes, leaving an 85 kDa, apoB-derived, lumen peptide which follows the default pathway for secretion.

quality-control system to ensure the production of a secretion competent particle. In this way, variation in the secretion of apoB can occur without any change in the concentration of apoB mRNA. Evidence suggesting an essential role for MTP in man was provided by Du *et al*. (1996), who detected truncated N-terminal apoB peptides, particularly an 85 kDa peptide, but not intact apoB100, in the plasma of six abetalipoproteinaemic patients. These peptides had arisen from degradation of translocation-arrested apoB due to the lack of MTP activity. The activity of MTP may thus govern the secretion of apoB in man. This assumes importance since, as mentioned earlier, VLDL is now thought to be an independent risk factor for CHD (Griffin & Zampelas, 1995), and overproduction of VLDL-associated apoB may contribute to familial combined hyperlipidaemia (Teng *et al*. 1986; Venkatesan *et al*. 1993). It has also been suggested that plasma apoB concentrations may be a better predictor of coronary atherosclerosis than total cholesterol or LDL-cholesterol (Sniderman *et al*. 1980). Obviously, the development of drugs which will inhibit apoB and therefore also triacylglycerol secretion, will be useful in the treatment of patients at risk of these clinical conditions. However, such therapy is not possible for the population as a whole, and it remains to be seen whether an understanding of the effects of specific dietary components on MTP gene expression can lead to nutritional strategies for the modulation of plasma lipid levels.

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Appendix

BiP:	binding protein resident in the lumen of the endoplasmic reticulum, acting as a chaperone to aid protein folding.
cis-, *trans*-Golgi:	the stacks of the Golgi apparatus can be subdivided into three distinct areas, *cis*-, *medial*- and *trans*-, through which newly-synthesized secretory proteins pass successively after leaving the rough endoplasmic reticulum.
COS-1, COS-7 cells:	SV40-transformed African green monkey (*Cercopithecus aethiops*) kidney cell lines.
Missense mutation:	a single base change in a gene which leads to an altered amino acid sequence in the mature protein.
Sf9 cells:	immortalized cells from the armyworm (*Spodoptera frugiperda*) pupal ovarian tissue.